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Mathematical Modeling of Population Dynamics of Unstable Plasmid-bearing Bacterial Strains under Continuous Cultivation in a Chemostat

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Abstract—We developed an approach to study the population dynamics of unstable recombinant bacterial strains cultivated in a chemostat. It is based on mathematical modeling of the distribution of cells bearing a different number of plasmid copies in the population. We analyzed the effect of a decreased selective advantage of plasmidless variants of the recombinant strain in the chemostat, which is related to a decreased number of plasmid copies in cells after long cultivation. It is shown that the time of plasmid half-elimination from the bacterial population in the chemostat at steady state, $T_{1/2}$, does not depend on the maximal number N of plasmid copies in the cells, and is determined solely by the average generation time g and the probability τ of losing a plasmid copy. We analyzed the dependence of the selective advantage of plasmidless bacterial variants cultivated in a chemostat on the expression genes cloned in plasmids, using as an example the *E. coli* recombinant strain Z905, whose pPHL-7 plasmids contain cloned genes of the luminescent system of marine luminescing bacteria *Photobacterium leiognathi.*

Key words: bacteria, plasmid-bearing strains, population dynamics, chemostat

INTRODUCTION

The majority of bacterial recombinant strains bearing genes cloned in plasmids are known to be often unstable both in batch and still more in prolonged continuous cultivation in a chemostat [1–4]. One should take into account at least the following facts when the population dynamics of unstable recombinant plasmid-bearing bacterial strains is described:

(i) plasmid segregational instability, when a portion of cells in the population lose their plasmids during reproduction;

(ii) instability of the plasmid genetic structure, when plasmids are retained in all cells but their form changes;

(iii) difference in the characteristics of the growth kinetics of the strain containing a certain number of plasmid copies as compared with the analogous plasmidless strain variants, the variants with the small number of copies, or the variants containing plasmids with altered structure.

A number of mathematical models that describe the population dynamics of plasmid-bearing bacteria under various cultivation conditions have been proposed [5]; among these of particular interest is the model of Levin and Stewart, which describes the segregational instability of the recombinant strains [6–7]. Basically, it describes the dynamics of populations of the plasmid-bearing n^+ and the plasmidless n^- cells in a chemostat, taking into consideration the probability of spontaneous plasmid loss in cell division and plasmid conjugal transfer between cells:

$$\begin{cases} \dfrac{dn^+}{dt} = \mu^+ n^+ - Dn^+ - \tau\mu^+ n^+ + \gamma n^+ n^-, \\ \dfrac{dn^-}{dt} = \mu^- n^- - Dn^- + \tau\mu^+ n^+ - \gamma n^+ n^-, \\ \dfrac{dS}{dt} = D(S_0 - S) - \dfrac{\mu^+ n^+}{Y^+} - \dfrac{\mu^- n^-}{Y^-}, \end{cases} \quad (1)$$

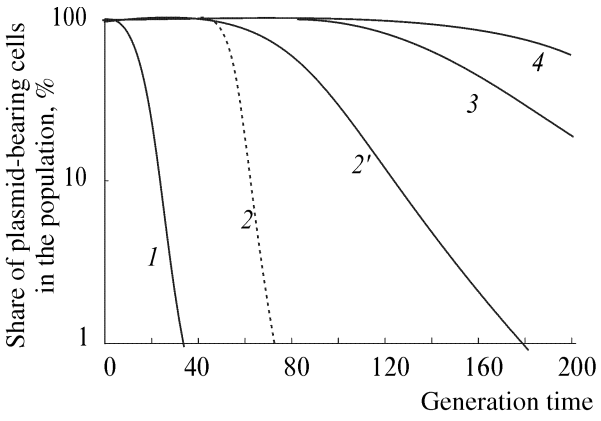

Fig. 1. Decrease in the selective advantage of the plasmidless recombinant strain during prolonged cultivation in a chemostat under the conditions nonselective for plasmid conservation: (*1*) competition of the plasmidless variant with the initial recombinant strain with the maximal number of plasmid copies in a cell, $N = 10$; initially, 0.01% of the plasmidless isogeneic cells is added into the chemostat (calculations were made according to model (5)); (*2*) spontaneous replacement of the initial strain with its plasmidless variant during prolonged cultivation: calculations according to the Lewin–Stewart model (1); (*2′, 3, 4*) spontaneous replacement similar to that in (*2*) but with calculations done according to the multicopy model (5); the maximal number of plasmid copies in a cell: (*2′*) $N = 10$; (*3*) $N = 20$; (*4*) $N = 30$. In all cases the following expression was chosen for the specific population growth rate: $\mu(x, S) = \exp(-0.5i/N)S/(1 + S)$, $\tau = 10^{-2}$, $D = 0.5$ h^{-1} (all coefficients are dimensionless).

where τ is the probability of formation of a plasmidless cell upon division; γ is the conjugal plasmid transfer rate parameter; D is the specific rate of media dilution in the chemostat; S, S_0 are substrate concentrations in the fermentor and in the incoming medium respectively; μ^+ and μ^- are the specific population growth rates for the plasmid-bearing and the plasmidless strains, respectively, which are functions of the substrate concentration limiting the growth: $\mu^+ = \mu^+_{\max} S / (K_s^+ + S)$, $\mu^- = \mu^-_{\max} S / (K_s^- + S)$, $\mu^+_{\max}$, $\mu^-_{\max}$ are the maximal growth rates, K_s^+, K_s^- are the Monod constants; and Y^+, Y^- are the coefficients of substrate consumption efficacy for the plasmid-bearing and the plasmidless strains, respectively.

Various experimental data on cultivation of plasmid-bearing strains obtained by now do not agree with this model [2, 3, 8]. In a number of cases, this is related to the fact that model (1) in essence pertains to the bacterial strains containing only a single copy of a plasmid (indeed, the model considers only two types of cell, the plasmid-bearing and the plasmidless one), which in principle is not true for the real recombinant bacteria, the majority of which contain many plasmid copies. Usually, the cell population contains a number of classes of cells bearing different numbers of plasmid copies [1–10]. Jones with his coworkers experimentally demonstrated [3] that the rates of displacement of a plasmid-bearing strain with a plasmidless strain are different for the cases of natural plasmid elimination during prolonged cultivation at steady state in a chemostat and after introduction of 0.1% of the isogeneic plasmidless cells into the population of plasmid-bearing bacteria. For such cases, however, the Levin–Stewart model (1) offers identical replacement rates (Fig. 1, curve *2*). One can suppose that during prolonged cultivation of multicopy recombinant plasmids in the chemostat the number of plasmid copies in the cells is reduced or the expression efficiency of genes cloned in the plasmids is decreased. If it is assumed that a greater number of plasmid copies in cells or a higher expression of plasmid genes decreases the specific rate of population growth, then the selective advantage of the plasmidless variants is reduced when the number of plasmid copies is small, and the curve of displacement of the plasmid-bearing strain with the plasmidless one should have a lesser slope (Fig. 1, curve 2′). In this paper, we present a mathematical model of plasmid segregational instability, which describes the population dynamics of the bacterial strains bearing many plasmid copies cultivated in selective and nonselective conditions, taking into account different expression levels of cloned genes.

FORMULATION OF THE MATHEMATICAL MODEL

Let us assume that at the initial moment the cell has N copies of the plasmid, and, with a certain probability τ_N, one plasmid copy is lost during cell division; correspondingly, with probability $(1 - \tau_N)$ all plasmids are replicated and divided equally between the daughter cells. Physically, this means that a cell of the class with i plasmid copies can move only to the nearest classes with $i + 1$ or $i - 1$ copies.

As the overall cell number is maintained constant in the chemostat steady state, to describe the

sequential loss of plasmids in cell division we can write down a continuity equation (see Appendix)

$$\frac{\partial F}{\partial t} + \mathrm{div}(\dot{x}F) = [\mu(x, S) - D]F, \qquad (2)$$

where F is the microbial population density; $\mu(x,S)$ is the specific propagation rate of the population; D is the specific rate of dilution in the chemostat; S is the concentration of the substrate limiting the microbial growth; x is the relative copy number of the plasmid DNA per cell, equaling i/N, where i is the current plasmid copy number in the cell: $i = 0, ..., N$ and N is the maximal plasmid copy number in the cell; $\dot{x}$ is the rate of plasmid flux from the variant with copy number x to the variant with copy number $x+ dx$. Given that the plasmids are segregationally unstable, the rate of plasmid loss can be taken proportional to their copy number in the cell and to the population specific growth rate, and thus write down

$$\dot{x} = -\tau\mu(x, S)x, \qquad (3)$$

where τ is the specific probability of the loss of one plasmid copy in cell division.

Uniting expressions (2) and (3), we arrive at an equation describing the dynamics of the plasmid copy number in the population of a recombinant strain:

$$\frac{\partial F}{\partial t} = [\mu(x, S) - D]F + \frac{\partial}{\partial x}[\tau x\mu(x, S)F], \qquad (4)$$

where $\frac{\partial}{\partial x} \equiv \mathrm{div}$ is a differential operator.

Supplementing equation (4) with an equation for the growth-limiting substrate S, we obtain a mathematical model of the population dynamics of a microbial plasmid-bearing strain with plasmid segregational instability in a chemostat:

$$\begin{cases} \dfrac{\partial F}{dt} = [\mu(x, S) - D]F + \dfrac{\partial}{\partial x}[\tau x\mu(x, S)F\,], \\ \dfrac{dS}{dt} = D(S_0 - S) - \displaystyle\int_0^1 \rho(x)\frac{\mu(x, S)F(x, t)}{y(x, S)}dx, \end{cases} \qquad (5)$$

where S_0 is the substrate concentration in the supplied medium; $y(x,S)$ is the efficacy of substrate utilization by the microorganism, which, in the general case, may vary depending on the plasmid copy number in the cells and on the substrate concentration in the medium; $\rho = di/dx$ is the state density.

SELECTIVE ADVANTAGE OF PLASMIDLESS VARIANTS IN THE CHEMOSTAT

Examination of the experimental data of Jones *et al.* [3] using the mathematical model (5) demonstrates that the varying rate of the displacement of plasmid-bearing cells by plasmidless ones can indeed be associated with the decline in the selective advantage of the plasmidless variants upon reduction of the plasmid copy number in the chemostat under conditions nonselective with respect to plasmid maintenance. On the other hand, a decrease in the average plasmid copy number per cell in multicopy recombinant strains must necessarily be observed upon long-term cultivation in the chemostat if the copy number is initially high enough ($N \propto 10$) (Fig. 1).

In the general case, the relative selective advantage of the plasmidless variant, which determines the selection pressure, would enter the distributed model (5) as $\alpha(x) = [1 - \tilde{\mu}(x) \,/\, \tilde{\mu}(0)]$, where $\tilde{\mu}(x)$ is the "apparent" specific growth rate for the variant with relative copy number x (cf. [5–8]). The $\tilde{\mu}(x)$ expression, among other things, takes into account the mortality, the influence of the efficacy of the expression of plasmid-cloned genes (ε) on the population specific growth rate μ, plasmid loss in cell division with a nonzero probability τ, and other effects that determine the "apparent" specific growth rate for the microbial population under study.

Using the structural model in the form (4), one can obtain an explicit equation for the relative selective advantage $\alpha(x)$ of the plasmidless variants accurate to the terms of the τ order:

$$\alpha(x) = 1 - \frac{\mu(x)}{\mu(0)} - \tau x\frac{\mu'(x)}{\mu(0)} \qquad (6)$$

where $\mu(x)$ is the "true" specific growth rate for the microbial population. In this equation, the third summand reflects the contribution of the probability τ of plasmid loss and the plasmid copy number x in the cell to the selective advantage, a feature basically beyond the Levin–Stewart model (1) which only describes the dynamics of single-copy strains. At certain

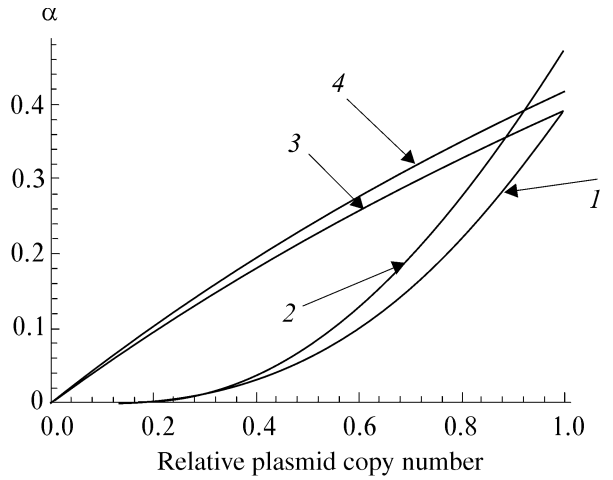

Fig. 2. Dependences of the relative selective advantage (α) of plasmidless variants on the plasmid relative copy number x in plasmid-bearing cells at different values of parameter τ (calculations by equation (6)): (*1*) for μ = = exp(–0.5 x^3), τ = 10^{-3}; (*2*) same but τ = 10^{-1}; (*3*) for μ = exp(–0.5 *x*), τ = 10^{-3}; (*4*) same but τ = 10^{-1}.

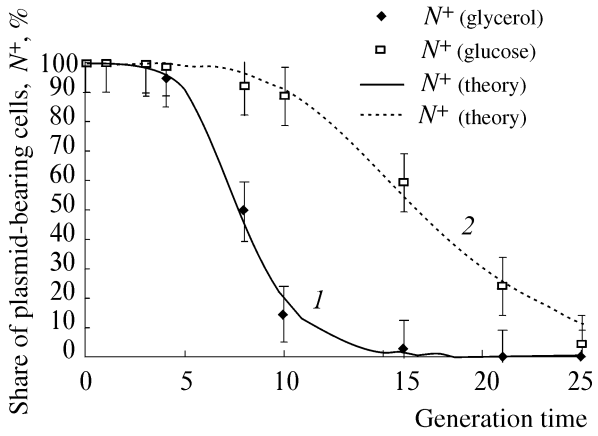

Fig. 3. Dynamics of the structure of the population of a recombinant bacterial strain *E. coli* Z905 (pPHL-7) in a chemostat (D = 0.1 h^{-1}): (1) glycerol and (2) glucose as rate-limiting substrates; points are experimental data, curves represent theoretical calculations according to the mathematical model (5).

x values this contribution can be quite sizable, depending on the specific growth rate gradient.

With the nonlinear dependence of the population specific growth rate on the plasmid concentration in the cells, there is a certain small plasmid copy number wherewith the selection pressure on such cells in the chemostat falls virtually to zero (Fig. 2). The curves for α(*x*) were generated using equation (6) at two values of parameter τ, and two expressions for the population specific growth rate: μ = exp(–0.5*x*) and μ = exp(–0.5x^3); the different power should reflect the different contribution of the efficiency of cloned gene expression and of the plasmid copy number to the population specific growth rate μ(*x*). In the latter case, at *x* << 1 the selective advantage of plasmidless variants becomes insignificant and, τ being small enough, low-copy variants can be maintained in a chemostat for long times, which has more than once been shown experimentally by us and other authors [8–11].

In our experiments, we assessed the duration of maintaining plasmid-bearing cells (or the rate of their elimination) in a chemostat (D = 0.1 h^{-1}) under nonselective conditions with different growth-limiting substrates: glucose or glycerol [8, 9]. The model object was an *Escherichia coli* strain Z905 containing a recombinant plasmid pPHL-7 with genes of the luminescent system of a marine light-emitting microorganism *Photobacterium leiognathi* cloned in pUC18 under the *lac* promoter. Changing the growth-limiting substrates allows one to vary the efficacy of expression of the cloned *lux* genes: on glucose the intensity of luminescence of the bacterial suspension is diminished by two or three orders of magnitude because the *lux* operon is subject to catabolite repression, which is abolished during growth on glycerol. The experimental data and the model calculations taking into account the influence of plasmid gene expression efficiency on the population specific growth rate as well as the probability of plasmid loss in cell division are collated in Fig. 3, demonstrating a good fit.

TIME OF HALF-ELIMINATION OF PLASMIDS FROM A POPULATION

In practice, it is often quite convenient to use, along with τ, γ, α(*x*), the instability parameter that characterizes the duration of the productive state of the population of the constructed recombinant strain. With approach (2) and model (5), this time interval is determined as follows.

Theorem. The half-elimination time for bacterial plasmids (i.e., the time interval in which half of all the plasmid copies are eliminated from the population) in a chemostat at low α(*x*) does not depend on the maximal plasmid copy number in the cells, and equals $T_{1/2} = g/\tau$, where g = ln2/D is the mean

generation time (time between two consecutive cell divisions) and τ is the probability of losing one plasmid copy in division.

Proof. The plasmid half-elimination time for unstable recombinant microbial strains is defined as the time interval in which half of all the plasmid copies are eliminated from the population. The overall number of plasmids in the population is written down as

$$L(t) = \int_0^1 xF(x,t)dx. \qquad (7)$$

Using definition (7) with allowance for the boundary condition $F(x = 1,\ t) = 0$, from the set of equations (5) we derive a mathematical model of the dynamics of the overall copy number of a plasmid in a bacterial population in a chemostat:

$$\frac{dL}{dt} = -DL + (1-\tau)\int_0^1 x\mu(x,S)F(x,t)dx, \qquad (8)$$

where the dynamics of plasmid distribution $F(x,t)$ is described by set (5).

In a chemostat close to a steady state, the following conditions are fulfilled at low $\alpha(x)$ [8, 12, 13]:

$$\begin{cases} \mu(x,S) \approx D, \\ \dfrac{dS}{dt} = 0. \end{cases} \qquad (9)$$

Then the dynamics of the overall copy number of a plasmid in a bacterial population according to (8) is determined by the equation

$$\frac{dL}{dt} = -\tau DL. \qquad (10)$$

Integrating the latter, we obtain that the time in which the initial number of plasmid is halved can be expressed as

$$T_{1/2} = \frac{g}{\tau}. \qquad (11)$$

Hence it follows that the half-elimination time for bacterial plasmids does not depend on the maximal plasmid copy number in the cells, and is determined only by the mean generation time and the probability of losing one plasmid copy in division. The results of calculations by model (5) confirming this conclusion for different values of the maximal plasmid copy numbers are displayed in Fig. 4.

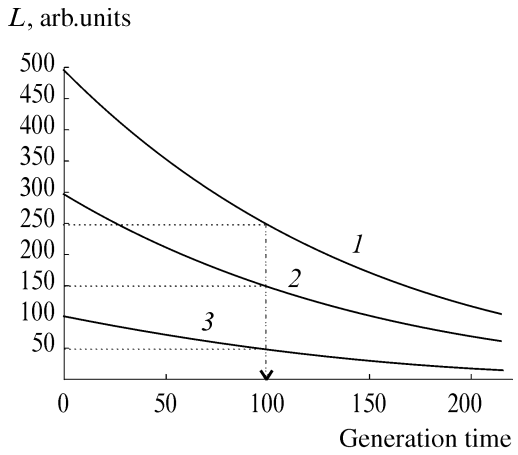

Fig. 4. Decline in the overall plasmid copy number in a bacterial population in a chemostat for different maximal copy numbers per cell (N): (*1*) 50, (*2*) 30, (*3*) 10; $\tau = 10^{-2}$; calculations performed according to the mathematical model (5). The arrow marks the plasmid half-elimination time. The ordinate is the overall number of plasmids in the population.

Thus, knowing the probability τ of a cell losing one plasmid copy, the expression for the half-elimination time can be used to determine the characteristic duration of the fermentation process till the moment when the productivity of an unstable recombinant strain drops by half because of plasmid loss. In this respect, the half-elimination time is an important characteristic of the population stability of gene-engineered microbial strains under intense cultivation.

CONCLUSION

In a more general case, to describe the population dynamics for several different plasmids in microbial cells, it is first of all necessary to form a vector of plasmids in the population:

$$\boldsymbol{x} = (x_1, x_2 \ldots x_j), \qquad (12)$$

where x_1 is the relative copy number of type 1 plasmids per cell, x_2 is the same for type 2 plasmids, *et seq*. Then, the properties of different types of plasmid are set in the form of differential equations describing the compatibility, segregational and structural instability, etc.:

$$\dot{\boldsymbol{x}} = \frac{d\boldsymbol{x}}{dt} = \boldsymbol{f}(x_1, x_2 \ldots x_j). \quad (13)$$

Substituting set (13) into the continuity equation (2) and adding the equation for the growth-limiting factor *S*, we arrive at the mathematical model of the population dynamics for a multicopy multiplasmid recombinant strain in a chemostat:

$$\begin{cases} \dfrac{\partial F}{\partial t} = [\mu(x, S) - D]F - \operatorname{div}(f)F - (f, \vec{\nabla}F), \\ \dfrac{\partial S}{\partial t} = D(S_0 - S) - \displaystyle\int_{x_i \ldots x_j} \frac{\mu(x, S)F}{y(x, S)} dx_1 \ldots dx_j. \end{cases} \quad (14)$$

Thus, analytical and numerical solution of set (14) allow description of the population dynamics of unstable recombinant microbial strains under various conditions of cultivation with allowance for the particular mechanisms of plasmid instability in the cell.

The approach to modeling the biological population dynamics based on the continuity equation has earlier been used by a number of authors to describe the distribution of organisms in certain features such as age or size [13–17]. The approach proposed in the present work basically enables one to describe the population dynamics for multicopy multiplasmid microbial strains also taking into account the incompatibility of particular plasmids and the different expression efficiency of the cloned genes.

APPENDIX

In the general case, equation (20 is derived from the law of conservation of the cell number for an arbitrary region of the phase space $V(dV = d^n\vec{x})$. In the process, it should be taken into account that, along with the flux across the boundaries caused by the change in the copy number with time, there are such events as cell birth and death, which can be regarded as sources $\mu(F,S)$ and sinks D distributed throughout the region considered. By the moment of time t, in the phase space volume considered there will be $\int F d^n\vec{x}$ individuals. The birth and death are covered by the following integral: $\int_0^t \int_V [\mu(\vec{x}, S) - D] d^n\vec{x} dt$. Differentiating these expressions in t, for any moment of time we obtain the law of conservation of the bacterial cell number in a chemostat:

$$\frac{d}{dt} \int_V F d^n\vec{x} = \int_V [\mu(\vec{x}, S) - D] d^n\vec{x}. \quad (15)$$

Transferring the operation of differentiation under the integral brings us to the following expression:

$$\int_V \frac{\partial F}{\partial t} d^n\vec{x} + \int_S (F(\vec{v})\vec{ds} = \int_V [\mu(\vec{x}, S) - D] d^n\vec{x}, \quad (16)$$

where $\vec{v} = \vec{v}[x, t]$ is the rate of plasmid transfer from the variant with the relative copy number $\vec{x}$ to the variant with $\vec{x} + d^n\vec{x}$. Making use of the Gauss–Ostrogradsky theorem, with standard operations and allowance for the arbitrariness of the region V, we obtain

$$\frac{\partial F}{\partial t} + \operatorname{div}(\dot{\vec{x}}\ F) = [\mu(\vec{x}, S) - D]F, \quad (17)$$

where $\operatorname{div}(\vec{v})$ is the divergence of vector $\vec{v}$.

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